

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Nielsen, et al.
Serial No. : 10/699,377
For : COMPOSITION
Filed : October 31, 2003
Examiner : Carolyn A. Paden
Art Unit : 1761
Confirmation No. : 3024

745 Fifth Avenue
New York, NY 10151

FILED VIA EFS-WEB
ON MAY 7, 2008

DECLARATION UNDER 37 C.F.R. §§ 1.131

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Jens Mogens Nielsen and Lars Høegh, declare and state that:

1. We are the named inventors on the above application (“the present application”), and are familiar with it and its prosecution.
2. Attached as Exhibit A are the claims as we understand are currently pending.
3. Attached as Exhibit B are the examples of the present application.
4. We understand that Loh (US 7,226,630), Smith (US 2004/0166204) and Gaonkar (US 7,229,654) have been cited against the present application. This Declaration is to confirm that the subject matter of the present invention was conceived and reduced to practice in the United Kingdom prior to the November 26, 2002 filing date of Loh, which reference has the earliest filing date of each of Loh, Smith and Gaonkar.
5. The invention as described by the pending claims provided as Exhibit A was conceived prior to November 26, 2002, as evidenced by a Technical Report authored by Jens

Mogens Nielsen, attached as Exhibit C. The Technical Report is entitled “Development of an analytical method for determination of water vapour permeability of lipid-based film and coatings” and describes the use of such coatings or barrier compositions as foodstuffs as claimed in the present application. Further, the Technical Report was authored and distributed in a GATT country, specifically Denmark.

6. The invention as described by the pending claims (Exhibit A) was reduced to practice prior to November 26, 2002, as evidenced by the Technical Report provided as Exhibit C, the Examples provided as Exhibit B, and the Notebook pages provided as Exhibit D. Specifically, prior to November 26, 2002, the experiments described in the Technical Report (Exhibit C) at page 18 were performed in a GATT country, namely Denmark. Those experiments are present in the pending application in the Examples (Exhibit B) and were recorded in the notebook pages provided herewith (Exhibit D).

7. The invention as described by the pending claims (Exhibit A) was conceived and reduced to practice prior to November 26, 2002, as further evidenced by the email and attachment from Mika Timmerbacka of Danisco, the assignee of the present invention, to David Alcock of D Young & Co. (Exhibit E) providing a description of a new application to be drafted and filed, which application, we are advised and therefore believe, was filed as United Kingdom patent application number 0301869.4, from which the present application claims priority.

8. That is, the invention as described by the pending claims in Exhibit A was conceived and reduced to practice prior to November 26, 2002, as evidenced by the Technical Report (Exhibit C), the Examples (Exhibit B), the notebook pages (Exhibit D), and the communication to from Mika Timmerbacka (Exhibit E), all of which relate to the conception and reduction to practice of the present invention in a GATT country prior to November 26, 2002.

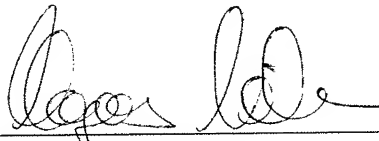
9. Accordingly, we are advised and therefore believe that the present application is entitled to antedate Loh, Smith and Gaonkar within the purview of 37 C.F.R. §1.131, and we request that Loh, Smith and Gaonkar be considered antedated and not available as prior art against the present application.

10. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code, and that such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

2/5-08
Date

5/5-08
Date


Jens Mogens Nielsen

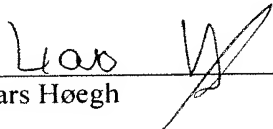

Lars Høegh

EXHIBIT A

PENDING CLAIMS

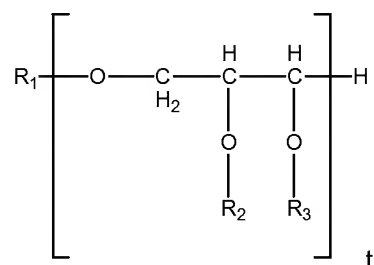
1. A multi-component foodstuff comprising:

- (a) a first food material;
- (b) a barrier composition; and
- (c) a second food material

wherein the first food material is coated with the barrier composition such that the barrier composition prevents or reduces movement of water from one food material to the other food material; and

wherein the barrier composition comprises

- i) a wax in an amount of 2 to 50 wt. % based on the composition
- ii) a compound in an amount of 50 to 98 wt. % based on the composition, having the formula



wherein t is an integer

wherein each R₁, R₂ and R₃ is independently selected from an acyl group or a hydrogen atom,

wherein at least one of R₁, R₂ and R₃ is H or an acyl group having from 2 to 6 carbon atoms (short acyl group)

wherein at least one of R₁, R₂ and R₃ is an optionally branched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms and an optional hydrophilic branch group (long acyl group).

2. The foodstuff according to claim 1 wherein the wax is selected from beeswax, candelilla wax, carnauba wax, jojoba wax, whale wax, paraffin wax, mineral wax, and microcrystalline wax.

3. The foodstuff according to claim 2 wherein the wax is beeswax.

4. The foodstuff according to claim 1 wherein at least one of R₁, R₂ and R₃ is an acyl

group having from 2 to 6 carbon atoms.

5. The foodstuff according to claim 1 wherein at least one of R_1 , R_2 and R_3 is a branched chain acyl group.

6. The foodstuff according to claim 5 wherein at least one of R_1 , R_2 and R_3 is a branched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms and a hydrophilic branch group.

7. The foodstuff according to claim 1 wherein at least one of R_1 , R_2 and R_3 is an unbranched acyl group.

8. The foodstuff according to claim 7 wherein at least one of R_1 , R_2 and R_3 is an unbranched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms.

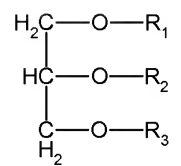
9. The foodstuff according to claim 1 wherein the one or more each optionally branched acyl group is unbranched.

10. The foodstuff according to claim 1 wherein t is from 1 to 10.

11. The foodstuff according to claim 10 wherein t is from 1 to 5.

12. The foodstuff according to claim 11 wherein t is 1 or 2.

13. The foodstuff according to claim 1 wherein the compound is of the formula



14. The foodstuff according to claim 1 wherein at least one of R_1 , R_2 and R_3 is H, and at least one of R_1 , R_2 and R_3 is an acyl group consisting of a saturated chain having 10 to 20

carbon atoms.

15. The foodstuff according to claim 1 wherein at least one of R_1 , R_2 and R_3 is an acyl group having from 2 to 6 carbon atoms, and at least one of R_1 , R_2 and R_3 is an unbranched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms.

16. The foodstuff according to claim 15 wherein two of R_1 , R_2 and R_3 are acyl groups having from 2 to 6 carbon atoms and wherein the other of R_1 , R_2 and R_3 is an unbranched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms.

17. The foodstuff according to claim 15 wherein the acyl group having from 2 to 6 carbon atoms is present in an average amount of no greater than 2 moles per mole of glycerol and esters thereof.

18. The foodstuff according to claim 15 wherein the unbranched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms is present in an average amount of at least 0.4 moles per mole of glycerol and esters thereof.

19. The foodstuff according to claim 18 wherein the unbranched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms is present in an average amount of from 0.9 to 2 moles per mole of glycerol and esters thereof.

20. The foodstuff according to claim 18 wherein the unbranched chain acyl group consisting of a saturated chain having 10 to 20 carbon atoms is present in an average amount of at least from 0.9 to 1 moles per mole of glycerol and esters thereof.

21. The foodstuff according to claim 15 wherein the average total amount of the acyl groups is 0.8 to 3.0 moles per mole of glycerol and esters thereof.

22. The foodstuff according to claim 1 wherein the chain of the long acyl group consists of a chain having 14 to 20 carbon atoms.

23. The foodstuff according to claim 22 wherein the chain of the long acyl group consists of a chain having 16 to 20 carbon atoms.

24. The foodstuff according to claim 1 wherein the short acyl group is an acyl group having from 2 to 5 carbon atoms.

25. The foodstuff according to claim 24 wherein the short acyl group is an acyl group having 2 carbon atoms.

26. The foodstuff according to claim 1 wherein the compound is an acetylated interesterification product of glycerol and an oil selected from fully hydrogenated, partly hydrogenated and non-hydrogenated fats and oils including palm oil, soy oil, rape seed oil, high erusic rape seed oil, sunflower oil, safflower oil, corn oil, cottonseed oil, lard, tallow, palm kernel oil, coconut oil, peanut oil, castor oil and fractions thereof.

27. The foodstuff according to claim 1 wherein the wax is present in an amount of 2 to 40 wt. % based on the composition.

28. The foodstuff according to claim 27 wherein the wax is present in an amount of 5 to 40 wt. % based on the composition.

29. The foodstuff according to claim 27 wherein the wax is present in an amount of 10 to 40 wt. % based on the composition.

30. The foodstuff according to claim 27 wherein the wax is present in an amount of 10 to 30 wt. % based on the composition.

31. The foodstuff according to claim 27 wherein the wax is present in an amount of 15 to 25 wt. % based on the composition.

32. The foodstuff according to claim 27 wherein the wax is present in an amount of approximately 20 wt. % based on the composition.

33. The foodstuff according to claim 1 wherein the compound is present in an amount of 60 to 98 wt. % based on the composition.

34. The foodstuff according to claim 33 wherein the compound is present in an amount of 60 to 95 wt. % based on the composition.

35. The foodstuff according to claim 33 wherein the compound is present in an amount of 60 to 90 wt. % based on the composition.

36. The foodstuff according to claim 33 wherein the compound is present in an amount of 70 to 90 wt. % based on the composition.

37. The foodstuff according to claim 33 wherein the compound is present in an amount of 75 to 85 wt. % based on the composition.

38. The foodstuff according to claim 33 wherein the compound is present in an amount of approximately 80 wt. % based on the composition.

39. The foodstuff according to claim 1 wherein the composition further comprises (iii) an auxiliary material selected from ionic emulsifiers and sorbitan esters.

40. The foodstuff according to claim 39 wherein auxiliary material is selected from citric acid esters, diacetylated tartaric acid esters of monoglycerides, sorbitan esters, and lecithin

41. The foodstuff according to claim 39 wherein the auxiliary material is present in an amount of from 0.1 to 1.0 wt. % based on the composition.

42. The foodstuff according to claim 41 wherein the auxiliary material is present in an

amount of from 0.25 to 0.75 wt. % based on the composition.

43. The foodstuff according to claim 41 wherein the auxiliary material is present in an amount of from 0.4 to 0.6 wt. % based on the composition.

44. The foodstuff according to claim 41 wherein the auxiliary material is present in an amount of approximately 0.5 wt. % based on the composition.

47. The foodstuff according to claim 1 wherein the foodstuff is selected from confectionery including sugar confectionery, chocolate, candy such as liquorice and water jellies, chewing gum, nuts; dairy products including cheese, whipped desserts, and ice cream; bakery products, either frozen or fresh and including bread, pizza, biscuits, crackers, cakes, pies; meat products including sausages, fish, ham, pork and beef, such as joints of pork or beef; fresh and dried fruit; and snacks.

49. A process for preparing a foodstuff, comprising coating a foodmaterial with a composition as defined in claim 1.

EXHIBIT B

EXAMPLES

Method

Water vapour permeability

Water vapour coefficients were measured using a SGA-100 Vapour Sorption Analyser from VTI Corporation 7650 West 26th Ave., Hialeah, FL 33016 USA. An aluminium cup of 2 cm in diameter was partly filled with deionised and de-aerated water leaving 3-5 mm free head space. On top of the cup a film consisting of the barrier composition was placed. The cup had a small flange to which the film was sealed with a high vacuum grease purchased from Dow Corning GmbH, 65201 Wiesbaden, Germany. Water phases other than pure water can be used. Pure water secures a water activity of 1 at any test temperature. Using a salty solution instead of water reduces the water activity and any desired water activity can be reached by adjusting the salt concentration or the type of salt in the water phase.

The cup was placed in the weighing chamber of the VTI instrument. The temperature and relative humidity was adjusted to test conditions and weight loss was registered and monitored over time. The test temperature could be varied between 5-80°C and relative humidity could be adjusted between 0 and 100%.

The film was prepared by melting the barrier composition to 80°C and carefully blending all ingredients to a homogeneous system.

A small (3 x 5cm) nylon filter, having no resistance to water migration and water evaporation, was placed on top of a hot microscope slide with a small pin in each end of the slide. The height of the pins could be varied. Hot and melted barrier composition was gently poured on top of the filter and another hot microscope slide was placed on top of the pins and the barrier composition was allowed to cool and crystallise. After complete crystallisation the glasses were gently removed and the film was transferred to the aluminium cup and sealed. The nylon filter provides a support matrix for the barrier composition. Another way of preparing the film was to immerse the nylon filter directly into the hot and melted barrier composition and allow it to crystallise before sealing it on top of the aluminium cup.

The monitoring of the weight loss due to water migration through the film and subsequent water evaporation was continued for 5-600 minutes.

The weight was then plotted vs. time leaving out the first 100 min, which was used to adapt to the specific measuring conditions in the beginning of each trial.

The slope of the plotted line was determined. The slope equals (area through which the water migrates) x (the difference in water vapour pressure across the film) x (the ratio between the permeability coefficient and film thickness). The film thickness was measured after each experiment with a micrometer using the average of 4 measurements. The area of the film was calculated from the diameter. The difference in water vapour pressure was controlled by the water phase composition in the aluminium cup and by the relative humidity in the weighing chamber of the VTI instrument.

With pure water in the cup the head space above the water surface has a relative humidity of 100%. The water vapour pressure in the head space was then 100% of the saturated water vapour pressure at the specific test temperature. Adjusting the relative humidity in the weighing chamber of the VTI instrument resulted in a water vapour pressure of 15% of the saturated water vapour pressure at the specific test temperature.

The overall driving force for water migration and evaporation from the film was then 85% of the saturated water vapour pressure at the test temperature.

10 to 15 films of each barrier composition were prepared and analysed as above. For all samples the ratio between the permeability coefficient and the film thickness was plotted against the reciprocal film thickness. The slope of this line equals the permeability coefficient.

Example 1

Distilled monoglyceride acetylated to 70% with a fatty acid composition mainly comprised of stearic acid and palmitic acid was analysed at 25°C with a water vapour pressure difference of 20.2 mmHg. The permeability coefficient was calculated to 995 mg mm / m² mmHg min.

Example 2

Distilled monoglyceride acetylated to 70% with a fatty acid composition mainly comprised of stearic acid and palmitic acid was analysed at 5°C with a water vapour pressure difference of 5.5 mmHg. The permeability coefficient was calculated to 721 mg mm / m² mmHg min.

Example 3

White beeswax was analysed at 25°C with a water vapour pressure difference of 20.2 mmHg. The permeability coefficient was calculated to 14 mg mm / m² mmHg min.

Example 4

Distilled monoglyceride acetylated to 70% and a fatty acid composition comprising mainly of stearic acid and palmitic acid was provided in an amount of 50% and heated to 80°C, 50% white beeswax was added, melted and carefully mixed with the acetylated monoglyceride by agitation. The films were prepared as described above using microscope slides. The permeability coefficient was measured and calculated at 5°C and yielded 20 mg mm / m² mmHg min.

Example 5

Distilled monoglyceride acetylated to more than 99% and a fatty acid composition comprised of mainly stearic acid and palmitic acid was mixed in a 90-10 ratio with white beeswax as described in Example 4 and analysed at 5°C. The permeability coefficient was calculated to 82 mg mm / m² mmHg min .

Example 6

Distilled monoglycerides acetylated to 70% with a fatty acid composition comprising of mainly stearic and palmitic acid was provided in an amount of 95% and mixed with 5% white beeswax as described in Example 4 and analysed at 5°C. The permeability coefficient was calculated to 115 mg mm / m² mmHg min.

Example 7

Distilled monoglyceride acetylated to 70% with a fatty acid composition comprising of mainly stearic acid and palmitic acid was provided in an amount of 90% and mixed with 10% white beeswax as described in Example 4 and analysed at 5°C. The permeability coefficient was calculated to 32 mg mm / m² mmHg min.

Example 8

A commercial product from Loders Croklaan Hogeweg 1, 1521 AZ, Wormerveer, Netherland, Coatbar A, which is a triglyceride composition was prepared as described in Example 4 without the use of beeswax and analysed at 5°C. The product was very brittle and easily formed cracks and holes. Furthermore it melted partly at ambient temperature and became soft. The permeability coefficient was calculated to 166 mg mm / m² mmHg min.

Texture

Samples of barrier compositions were melted and carefully mixed at 80°C and poured into cylindrical glass beakers 6 cm in diameter and approximately 5 cm tall. After 3 days of storage at the test temperature the consistency of the samples were analysed by use of a Texture Analyser TA-XT2 from Stable Micro Systems, Vienna Court, Lammas ad, Godalming, Surrey GU7 1YL, UK, equipped with a 2 mm P2 DIA CYLINDER STAINLESS probe. The probe penetrated the samples in one cycle with a prespeed of 2.0 mm/s and a penetrating speed of 0.5 mm/s. Distance was set to 10.0 mm and post speed was 2.0 mm/s. Trigger weight was 3.0 g.

Each measurement provided a graph showing the force in gram required to penetrate the samples vs. time. All curves exhibited the same basic shape with an initial steep raising and an inflection point where the slope of the graph decreases.

The measured force in gram at the inflection point (from now on called inflection force) was used for comparison between the samples. A decrease in the measured force at the deflection point was seen for softer and more elastic compositions.

Example 9

The inflection force for different barrier compositions are shown below:

Barrier composition	Inflection force g at 5°C	Inflection force g at 20°C
100% Acetylated monoglycerides from ex 5	165	
90% Acetylated monoglycerides from ex 5 + 10% white beeswax	500	
80% Acetylated monoglycerides from ex 5 + 20% white beeswax	1000	
100 % White beeswax	4000	2200
20% Acetylated monoglyceride from ex 1 + 80% white beeswax	3400	
50% Acetylated monoglyceride from ex 1 + 50% white beeswax	2700	
60% Acetylated monoglyceride from ex 1 + 40% white beeswax	2000	
70% Acetylated monoglyceride from ex 1 + 30% white beeswax	1700	
80% Acetylated monoglyceride from ex 1 + 20% white beeswax	1400	700
90% Acetylated monoglyceride from ex 1 + 10% white beeswax	900	400
100% Acetylated monoglyceride from ex 1 +	340	200

Solid Fat Content

Mixtures of acetylated monoglycerides listed in Example 9 and white beeswax in different proportions ranging from pure beeswax to pure acetylated monoglycerides were tempered according to IUPAC 2.150a standard tempering method and the SFC (solid fat content) was measured on Bruker NMS 120 Minispec NMR Analyser, 762287 Rheinstetten , Germany, at 5°C and 20°C. The results show a linear relationship between mixing ratio of the acetylated monoglycerides and the white beeswax and the SFC without any sign of eutectic effect, which would have seriously damaged the water vapour barrier properties. The SFC measurements

also showed that especially the acetylated monoglyceride listed in Example 1 mixed with white beeswax only loses approximately 2% point of the total solid fat content when heated from 5°C to 20°C. The barrier is then able to persist temperature fluctuations.

Barrier composition	SFC at 5°C	SFC at 20°C
100 % White beeswax	93.8	90.6
20% Acetylated monoglyceride from ex no 1 + 80% white beeswax	94.0	89.9
50% Acetylated monoglyceride from ex 1 + 50% white beeswax	92.9	89.8
60% Acetylated monoglyceride from ex 1 + 40% white beeswax	92.5	89.5
70% Acetylated monoglyceride from ex 1 + 30% white beeswax	92.2	89.6
80% Acetylated monoglyceride from ex 1 + 20% white beeswax	91.9	89.5
90% Acetylated monoglyceride from ex 1 + 10% white beeswax	91.5	89.3
100% Acetylated monoglyceride from ex 1	90.9	88.9

Application

Example 10

A model application system was prepared by spraying approximately 400 mm on a square cracker, namely a Barber Cream Cracker, produced by The Horizon Biscuit Company Ltd. Pasture Road, Moreton, Merseyside CH46 SE England. The melted 80°C hot barrier compositions were sprayed on the cracker in a 4-run-cycle on both sides on a conveyor belt. Between each cycle the cracker was turned 90°. Prior to the heating the cracker was preheated for a few seconds in 50-60°C hot air. After adapting to ambient room temperature for 1 hour a carrageenan gel was placed on top of the cracker. The gel had a water activity of 1 and it was prepared by dissolving 0.2% CaCl₂ and 0.2% KCl in distilled water and adding 3% carrageenan, during slow agitation. After heating the water to 85-90°C 0.1% Na-benzoate was

added. After cooling to less than 75°C 0.6% citric acid solution was added (50% w/w). The liquid gel phase was poured in glass beakers and stored at 5°C.

The water activity of the cracker was 0.2 at 22°C.

Before applying the gels on the top of the crackers all crackers were weighed (zero-value). The crackers with gels placed on the top were stored at 5°C. Over the following days 10 crackers were weighed each day after gentle removal of the gel. The gel did not stick to the cracker.

For each cracker the respective zero-value was subtracted from the daily weight and the weight gain was calculated as the average of the 10 zero-value corrected measurements.

A barrier formulation as described in Example 1 was used and the weight gain (gram water per gram cracker) due to water migration was

Day 1	0.8 g
Day 2	1.5 g
Day 4	2.2 g
Day 7	2.8 g
Day 14	3.5 g

A cracker with no barrier system applied but treated similarly resulted in the following results:

Day 1	1.8 g
Day 2	2.2 g
Day 4	3.2 g

Example 11

A barrier system as described in Example 3 was tested as described in Example 10. The following weight gain due to water absorption was recorded.

Day 1	0.9 g
Day 2	1.6 g

Day 4	2.8 g
Day 7	3.5 g
Day 10	4.1 g
Day 14	4.3 g

Example 12

A barrier system as described in Example 4 was tested as described in Example 10 and it provided the following weight gain results due to water migration:

Day 1	0.1 g
Day 4	0.5 g
Day 7	1.4 g
Day 10	2.0 g
Day 14	2.6 g

Example 13

A barrier system made of 20% pure white beeswax and 80% acetylated monoglycerides with a fatty acid composition comprising of mainly stearic and palmitic acid was applied on the crackers as described in Example 10. The weight gain due to water migration was:

Day 2	0.1 g
Day 4	0.2 g
Day 8	0.5 g
Day 14	0.8 g

Example 14

A barrier system as described in Example 1 was applied in 150 mm thickness as described in Example 10 followed by application of a new barrier layer on top of the first one. The second barrier system was similar to the barrier system described in Example 13. The weight gain due to water migration was:

Day 2	0.1 g
Day 4	0.2 g
Day 8	0.3 g
Day 12	0.5 g
Day 16	0.6 g

Example 15

A barrier system as described in Example 1 was applied on 75 mm thickness as described in Example 10 followed by application of 150 mm of the barrier system described in Example 13. On top of the second barrier layer new 75 mm layer of the barrier system described in Example 1 was applied. The weight gain due to water migration was:

Day 1	0.1 g
Day 5	0.2 g
Day 12	0.4 g
Day 16	0.5 g

Example 16

Distilled monoglyceride acetylated to 70% with a fatty acid composition comprising mainly stearic and palmitic acid was mixed in an amount of 80% with 20% white beeswax as described in Example 4. The barrier system was applied on crackers as described in Example 10 in 300 mm with the change that the applied gel had a water activity of 0.62.

The gel was prepared by premix 2% GRINDSTED^{RTM} PECTIN CF 140B, from DANISCO A/S, Brabrand, Denmark, with 3% sugar and 20% water. The premix was added to a boiling mixture of 66% white sugar syrup 80% SS and 25% sugar. The mixture and the premix were boiled until a weight of 100% followed by addition of citric acid.

The weight gain due to water migration was:

Day 1	0.01 g
Day 2	0.04 g

Day 5	0.05 g
Day 8	0.11 g
Day 16	0.17 g

Example 17

A barrier system as described in Example 7 was prepared with the change that standard soy lecithin, was melted into the original barrier mixture in the following concentrations: 0.05%, 0.1%, 0.2% and 0.3%. The texture of the barrier system was evaluated as described in "Texture" with the change that the force at 10 mm penetration was registered instead of the inflection force.

Lecithin	Force g at 10 mm 5°C
0%	2488
0.05%	2752
0.10%	2592
0.20%	2406
0.30%	2971

Example 18A

Acetylated distilled monoglycerides as described in Example 4 were mixed with 20% carnauba wax and melted and mixed at 90°C followed by cooling and crystallisation at room temperature. After further 24 hours storage at room temperature the mixture was heated to 60°C and the yield stress was measured at 60°C on a Reological StressTech reometer using a CC 25 CCE measuring probe. The applied stress sweep clearly showed the yield stress and the subsequent break down of the internal structure when the applied stress was higher than the yield stress. The yield stress curve is shown in Figure 1 (Figure A). The yield stress was analysed to 0.1 Pa at 60°C.

Example 18B

Acetylated distilled monoglycerides as described in Example 18A were mixed and melted with 30% carnauba wax at 90°C and treated similar as in Example 18A except that the yield stress

measurement was performed at 70°C. The yield stress curve is shown in Figure 2 (Figure B). The yield stress was analysed to 0.1 Pa.

Comments - The internal structure of the barrier system breaks down in both cases (Examples 18A and 18B) at a shear stress of 0.1 Pa. As long as the structure is intact the sample resists to flow and it is able to overcome increased stress applied from the instrument. This increased resistance to flow is measured as an viscosity increase. As soon the structure breaks down the measured viscosity decreases rapidly. This takes place at 0.1 Pa.

Example 19

A barrier system as described in Example 13 was applied to crackers at a thickness of 400 mm in the manner described in Example 10. A gel, prepared in accordance with Example 16, having a water activity of 0.79 was placed on top of the crackers. The weight gain of the crackers due to water migration at 20°C was found to be:

Reference after one day of storage:	10% weight increase.
Sample with 400 mm barrier system:	2.1% weight increase after 2 days of storage
Sample with 400 mm barrier system:	6 % weight increase after 7 days of storage
Sample with 400 mm barrier system:	9 % weight increase after 14 days of storage

EXHIBIT C

TECHNICAL REPORT

CONFIDENTIAL

TITLE Development of an analytical method for determination of water vapour permeability of lipid-based film and coatings		TR 6126
NAME AND LOCATION OF AUTHOR Mogens Nielsen, PFS		PAGE 1 OF 224 PAGES
DATE OF ISSUE Redacted	REFERENCE SBP, LIV, JTG, EVH	PROJECT NO. 2902
LAB BOOK REFERENCE See appendix 2		
KEY WORDS Water vapour permeability, edible coating, edible film, water barrier, analytical method for determination of water permeability in lipid-based film and coating.		
DISTRIBUTION: Original: DOM (DCDK) Innovation Management: HSP/HZK (DCDK), AJM/JDM (DCIC) Patents: ABL (DICC) Strategic Business Unit: JHL Master Project Manager: Others: LAH, SKO, ILP, ANG, FVS (DCDK), JRD (DCUSK)		

ABSTRACT

A method for measuring water vapour permeability of lipid coating and film has been developed using the automated water vapour sorption analyser, VTI SGA-100, which is able to register any weight change for a system in controlled temperature and humidity conditions.

The film or coating is prepared either by spraying it on a Ca-silicate tablet or forming a self-supporting film on a piece of filter cloth.

The tablets need conditioning at 54% relative humidity prior to both the spraying process and the sorption analysis. During measurement the tablet is exposed to a sudden increase in relative humidity, and based on the rate of water adsorption the permeability coefficient of the coating layer can be calculated.

For self-supporting films the film is placed on a cup containing water and the weight loss through the film in controlled temperature and humidity conditions is registered. From the rate of weight loss the permeability coefficient of the film can be calculated.

The calculations are automated by use of software programmed in Microsoft Excel.

The tablet method is much more time-consuming and complicated to run. It involves a spraying process, long-term humidity conditioning and long analytical time in the sorption analyser. Furthermore the calculations are much more complicated.

The cup method is faster both in respect to film formation and analysis. Up to three samples per day can be analysed against only one per day for the tablet method.

The results of the measurement is the permeability coefficient expressed as the amount of water passing through a film with a certain thickness per film area per pressure difference across the film per time unit ($\text{mg my} / \text{m}^2 \text{ mmHG min}$). The coefficient can be used to evaluate and compare the effectiveness of any potential coating material.

FOR INTERNAL USE ONLY - DO NOT COPY!

INTRODUCTION

Resistance against water vapour transport is one of the most important properties for an edible coating used in food application. Water influences and controls texture, appearance and shelf life of many products and especially in combined foods where one part of the food has very different water activity than the rest of the food product e.g. sandwiches with cheese or crackers with jam or cheese, water transport from one part to the other will cause a severe damage to the overall quality of the food. In such cases a film barrier between the different food components may be required.

DC does not currently have any standardised method to measure water vapour permeability of coatings. Knowledge about water vapour permeability of DC products such as coatings will provide very basic information useful in application and development of potential coatings and it might save expensive and time-consuming application tests. Furthermore a catalogue with permeability data would be very useful in the dialogue with costumers.

Two different methods for measuring water vapour permeability have been developed and tested. The first method (cup method) requires a film or coating layer that is free-standing or self-supporting (such as polymer film). The second method (tablet method) is for coating materials that are not self-supporting, but must be applied on a material (for instance by spraying). The latter method is however much more complicated as this involves a spraying process and the required calculations are significantly more complicated as the water uptake and A_w in the object on which the coating has been sprayed also needs to be taken into account. Due to the more complicated method the first method has therefore also been modified so it can be used for non-self-supporting films.

This is done by moulding the material on a piece of plastic polymer filter that works as a skeleton.

LITERATURE

Enrobing food with a lipid-based material has long been known to prevent water-loss during storage of e.g. fruits. The lipid compounds include natural waxes, surfactants and acetylated monoglycerides. Acetylated monoglycerides possess unique properties due to the very wax-like consistency in molten form. The film made from acetylated monoglycerides is very flexible. J. J. Kester [5] reports that acetylated monoglyceride can be stretched 8 times compared to many other lipids in the solid state, which can only be stretched 2%. This phenomenon should be due to the alpha stability of acetylated monoglycerides.

The barrier properties of acetylated monoglycerides against water migration improve with increasing acetylation, because of the removal of the free hydroxyl group that otherwise interacts with the migrating water. J. J. Kester [5] further reports that the permeability of water through acetylated monoglycerides depends not only on the driving force (difference in water pressure across the film), but also on the actual water pressure on both sides. This is illustrated by an example: Keeping the pressure at 0 mmHg on the one side of the film while increasing the pressure from 6.2 mmHg to 18.2 mmHg on the other side increased the permeability 3 times as expected, but subsequently increasing the low pressure side to 14.1 mmHg further increased the permeability 3 times. This is seen for hydrophilic films, which do not obey Fick's law (see THEORY AND MATHEMATICAL MODEL). Therefore the permeability data for hydrophilic films must also be followed by information on the actual water vapour pressure used during the measurements. Hydrophobic films, which do not interact with migrating water molecules, follow Fick's law.

Natural waxes are significantly more resistant to water migration than acetylated monoglycerides. J. J. Kester [5] reports 10 times less water permeability and argues that this is due to the high content of high-melting components and the absence of polar groups in the wax.

The cup method provides a coefficient that describes and relates to the coating material alone. The tablet method involves a spraying process relating it much more to real life application of coatings.

GRINDSTED® ACETEM 70-00 and White Bee Wax have been used as test material. The permeability coefficients reached correspond closely with the ones found in literature. For GRINDSTED® ACETEM 70-00 a value of 995 mg my/ m² mmHg min at 25°C was found compared with 782 mg my/ m² mmHg min for an acetylated monoglyceride of unknown composition at 21°C in the literature. For White Bee Wax the permeability coefficient was analysed to 14.1 mg my/ m² mmHg compared with 1.74 mg my/ m² mmHg for a Bee Wax in the literature. As Bee Wax is a natural product, the one referred to in the literature presumably differs from the one used in this project.

CONCLUSION

The method provides new possibilities for DC to focus on development of edible coatings by reducing time-consuming application tests, quantifying the barrier properties, providing basic barrier data for customer contacts and development work. Furthermore, the theory behind the method also describes ways to predict shelf life of foods in specified conditions and also the influence of storage temperature on the shelf life relating to water migration can be estimated based on the new method.

Listed below is an overview of the method with advantages and disadvantages:

Method	Film formation	Measurement	Advantages	Disadvantages
Tablet	Spraying	Adsorption during a %RH step	Relates closer to real application	Time consuming Difficult calculations Difficult spraying process
Cup	Moulding	Desorption at constant %RH	Material coefficient Easy film formation Fast analysis %RH can be adjusted on both sides of the film	

In future, the cup method will be used as the first choice. The advantages are large compared with the tablet method.

Based on the cup method an estimate of the permeability coefficient at one specific temperature includes 10 measurements with a total labour cost of approx. 10 hours. The temperature range is 5°C-80°C. Humidity range is 5%-95%.

Film thickness can be adjusted between approx. 100-700 µm depending on the permeability parameters.

In principle the cup method can also be applied on plastic films, but it might be limited by the very small film area reducing the total water loss to a minimum for very tight films.

However, waxes are often not very flexible and that reduces the practical effectiveness of the wax as a water vapour barrier where small holes or cracks lead to a significantly higher permeability than expected from the size of the pinhole.

J. J. Kester et al [7] has measured the water vapour permeability of different polymorphic forms of triglycerides and found that the alpha form has the highest resistance to water migration followed by the beta form. The beta-prime form has the lowest resistance. Increased density of the barrier improves the barrier properties. However there is only limited increase in the density during an alpha-beta-prime transformation. Instead the lipid loses plasticity originating from the hexagonal packing geometry in the alpha-form rendering the film less perfect with crystal boundary defects.

The transformation from the beta-prime to the beta form is followed by a significant increase in density. This will increase the resistance to water migration, but it still does not revert to the level as for alpha crystals.

Francesca et al. [6] reports that resistance against water migration increases with increasing fatty acid chain length of lipids. The same behaviour is seen with increased saturation of the fatty acids. They also report that the internal organization of the barrier layer influences the resistance of water migration. A homogeneous structure exhibits better barrier properties than an inhomogeneous one.

THEORY AND MATHEMATICAL MODEL

A component can diffuse through a coating or film in two ways. The component can either pass the barrier through small pinholes or cracks or it dissolves in the coating or film and then migrates to the opposite surface. Therefore, a concentration gradient exists in the film and the permeability depends on two factors, the solubility of the component in the film and the migration of the component in the film.

The overall capability of a film or coating to resist water diffusion thus depends on the solubility – and diffusion coefficients of water in the material – but also on the ability of the coating and film to form a layer without holes and cracks. This signifies that a flexible and elastic coating would be more useful than a brittle one.

The permeability P is defined as the product between the Diffusion coefficient (D) and the Solubility coefficient S :

$$P = D * S \quad \text{Eq. 1}$$

In dilute systems Henry's law defines the Solubility coefficient as :

$$C_a = S * P_a \quad \text{Eq. 2}$$

C_a is the concentration of component a in a solution and P_a is the partial pressure of component a above the solution.

During steady state vapour flow through a film, Fick's law of diffusion applies:

$$J = \frac{D * dC_a}{dX} \quad \text{Eq 3}$$

D is the diffusion coefficient and dc/dx is the concentration gradient of the migrant in the film.

J is the flux of component a. X is the film thickness.

Eq 1 to 3 can be combined and assuming a steady vapour flow through the film:

$$J = \frac{P}{S} * \frac{dCa}{dX} \Rightarrow$$

$$J = \frac{P}{S} * \frac{S * dPa}{dX} \Rightarrow$$

$$J = \frac{P}{X} * (Pa2 - Pa1) \quad \text{Eq 4}$$

(Pa2-Pa1) is the difference in partial pressure of the migrant across the film.

J is defined as m / (A * t) where m is the amount of migrant passing through the area A in time t. Exchanging J in eq. 4 with this expression yields:

$$\frac{m}{A * t} = \frac{P}{X} * (Pa2 - Pa1) \quad \text{or} \quad \text{Eq 5}$$

$$P = \frac{m * X}{A * t * (Pa2 - Pa1)} \quad \text{Eq 6}$$

Eq. 6 defines the permeability coefficient P with the following units : g H2O mm /m2 mmHg day

Eq. 5 can be written as

$$m = \frac{P}{X} * A * (P_e - P_i) * t \quad \text{eq. 7}$$

where m = amount of water transferred through the film

P = Permeability coefficient

A = surface area of the film

Pe - Pi = water vapour pressure difference across the film.

Pe-Pi is the driving force.

t = time

X = film thickness

If the water vapour pressure on both sides of the film is constant, there is a linear relationship between m and t, and P/X can be calculated from the slope of the line for the plot m VS t.

Equation 7 applies for free-standing, self-supporting films where the humidity can be controlled on both sides of the film. If the film is not free-standing and self-supporting, the film must be coated on a material. In that way the humidity of the inner side of the film cannot be held constant, and consequently (Pe- Pi) in eq. 7 changes during time. This means that Pi is controlled by the way the material adsorbs water as described by the water vapour adsorption isotherm for the material. As the calculation now requires knowledge of the adsorption isotherm the mass change due to water transport is now expressed on basis on the dry weight of the sample in equation 8.

$$\frac{dm}{W_s} = \frac{P}{x} * A * (P_e \div P_i) * dt \quad \text{eq. 8}$$

m: water vapour absorption

Ws: dry weight of the sample

P= permeability of the film

X=thickness of the film

A= effective area of the film through which the water transport takes place.

Pe: Equilibrium pressure of the water vapour at the specific temperature.

The equilibrium adsorption isotherm measured at a specific temperature relates the total water adsorption based on the dry weight with the partial water vapour pressure expressed as the % relative humidity (%RH) or Aw (%RH/100). Many mathematical models have been derived to describe the isotherm and it is generally accepted that the GAB equation applies very successfully to a large number of foods in the range of 0 to 90 %RH. The GAB equation was developed on principles of statistical mechanics and kinetics and the parameters in the equation have physical relevance. However as the equation contains 3 constants and two variables it needs 5 equations to be solved. This makes it very difficult to use in eq. 8. Instead a linear model can be used for a part of the isotherm as seen in fig. 1. Using the linear model for the adsorption isotherm ($P_e \div P_i$) can be expressed as

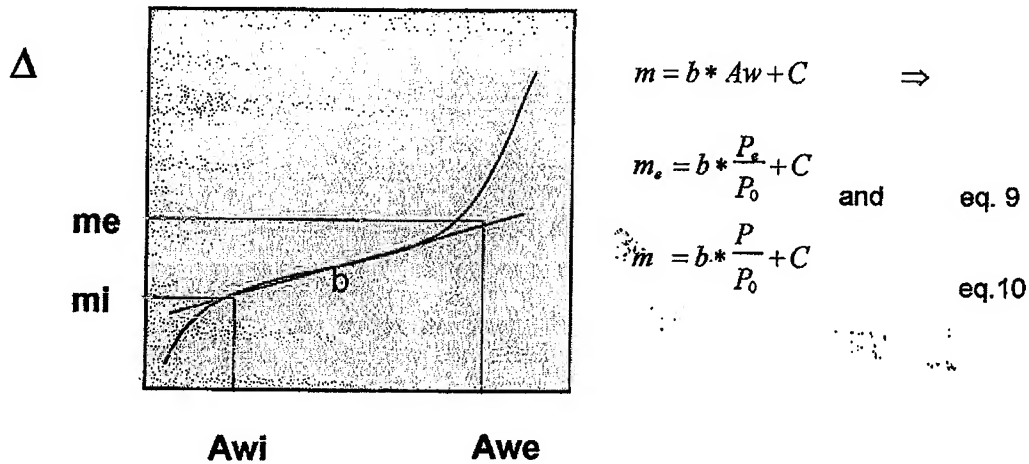


Fig. 1. Linear approximation of the adsorption isotherm.

Equation 10 subtracted from equation 9 yields:

$$m_e \div m = \frac{b}{P_0} * (P_e \div P) \Rightarrow$$

$$(P_e \div P) = \frac{P_0}{b} * (m_e \div m) \quad \text{eq. 11}$$

Substituting ($P_e \div P$) in eq. 8 with the derived expression in eq. 11 yields:

$$\frac{dm}{Ws} = \frac{P}{x} * A * \frac{P_0}{b} * (m_e \div m) * dt \Rightarrow$$

$$\int_{m_i}^m \frac{1}{m_e - m} * dm = \frac{P}{x} * \frac{A}{Ws} * \frac{P_0}{b} * \int_0^t dt \Rightarrow$$

$$[-1(LN[m_e - m])]_{m_i}^m = \frac{P}{x} * \frac{A}{Ws} * \frac{P_0}{b} * t \Rightarrow$$

$$LN \left[\frac{m_e - m_i}{m_e - m} \right] = \frac{P}{x} * \frac{A}{Ws} * \frac{P_0}{b} * t \quad \text{eq. 12}$$

Equation 12 describes the kinetics during water vapour adsorption for a particular substance when it is coated or wrapped in a film. In controlled conditions, equation 12 can be used to calculate $\frac{P}{x}$.

m_i is the initial water content, m_e is the equilibrium water uptake at the external relative humidity calculated on basis of the linear working isotherm, b is the slope of the linear working isotherm for the food, A is the total area of the film or coating, P_0 is the saturated water vapour pressure at the test temperature and Ws is the dry weight of the test sample. In this project calcium trisilicate in tablet form was used. The geometry and weight of the tablets is expressed in the A/W ratio and is controlled. P_0 is given by the test temperature of 25°C. b depends on the adsorption isotherm for calcium trisilicate at 25°C and must be measured separately. m_e is given by the linear working isotherm and is the equilibrium water uptake at the controlled external relative humidity at 25°C.

Plotting $LN \left[\frac{m_e - m_i}{m_e - m} \right]$ as function of time yields a straight line from which the slope P/X then can be calculated.

Note that equation 12 is only valid in the range of m and A_w where the true adsorption isotherm is linear as described by the linear working isotherm. If using equation 12, these limitations should be monitored when selecting data for calculation of P/X .

In both eq.7 and eq.12 the measured P/X originates from the total permeability of not only the permeability through the coating or film, but also from the resistance to water uptake in Ca-silicate tablet and the resistance to water evaporation from the cup. These factors can be taken into account by measuring the permeability coefficient for non-coated tablets and the time-depending water evaporation with out resisting film. As the reciprocal permeability coefficients are additive, the true permeability data for the film or coating can be found using eq.13:

$$\frac{1}{\left(\frac{P}{X}\right)_s} = \frac{1}{\left(\frac{P}{X}\right)_e} + \frac{1}{\left(\frac{P}{X}\right)_f} \quad \text{eq.13}$$

$\left(\frac{P}{X}\right)_s$ = Total P/X calculated from eq.7 or eq.12

$\left(\frac{P}{X}\right)_e$ = P/X measured and calculated for non-coated tablets and for measurements without barrier layers.

$$\left(\frac{P}{X}\right)_f = \text{True } P/X \text{ for the coating layer.}$$

The relationship between the above three P/X -values can be illustrated by the following figure:

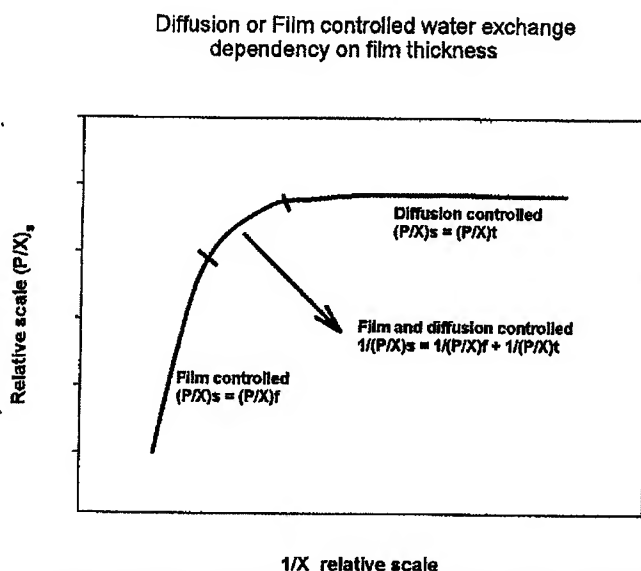


Fig. 2 Illustration of diffusion and film controlled water exchanged.

In true film controlled conditions there is no need to take the diffusion into account as the resistance to water migration is much larger for the film than for the diffusion in for instance the tablet.

However for film that does not resist water migration or for very thin film, the resistance to water migration originating from the diffusion process must be taken into account as described by equation 13.

To summarise the above, equation 7 is used for self-supporting films, equation 12 is used when the coating is sprayed on a material and the true permeability data P/X is calculated from eq.13.

Finally, all measured and calculated $\left(\frac{P}{X}\right)_f$ data are plotted VS reciprocal film thickness providing a straight line which slope equals the permeability coefficient P .

EXPERIMENTAL

Methods for self-supporting films and none-supporting films have been established and tested. The principle for the self-supporting films is that the film is placed on top of a small cup with a known area. The cup contains water securing a head space with 100% RH. The cup is then placed in a balanced weight in a controlled temperature and relatively humidity atmosphere (25°C and 15% RH). The weight loss of the cup was recorded as function of time and P/X was calculated from equation 7.

For none-supporting films the coating material is sprayed on Ca-silicate tablets with a known surface area. The tablet was then placed in the balanced weight equipment and exposed to a sudden increase in RH during which the weight gain versus time was monitored. P/X was calculated from equation 12.

None-supporting films (spraying method):

Ca-silicate tablets were produced by Glostrup Apotek. Ca-silicate tablets were chosen as this material is able to adsorb sufficient water and it can be formed to tablets without any processing aids. Furthermore the tablets are mechanically and temperature stable for the following coating.

Before spraying the barrier on the tablets the tablets were conditioned at 54% RH for at least 2 days.

The reason for this was that Ca-silicate contains CaO, which binds water strongly as Ca(OH)₂. Ca(OH)₂ can not be removed in the initial drying process that starts the measurement. Exposure to 54% RH secures saturation of the CaO.

Conditioning:

At least two days in an atmosphere with approx. 54% RH at room temperature. 54% RH was obtained by dissolving/adding more than 670 g Mg (NO₃)₂ to 100 ml water (saturated solution) and placing the tablets in the head space.

Spraying:

Spraying was done with a BuCHI mini spray dryer type: B-191 F-nr. 1362098

The emulsifier was melted at hold at a temperature of 65°C for GRINDSTED® ACETEM 70-00 and 85°C for the bees' wax. Agitation 400 rpm.

Air inlet temperature = 120°C. Aspirator = 50%. Pump speed = 20%. Air flow = 200 ml/s. As shown in fig. 3 the nozzle was held in an angle of approx. 60° to coat the side of the tablets. The tablet was rotating with 40 rpm. Both sides were coated.

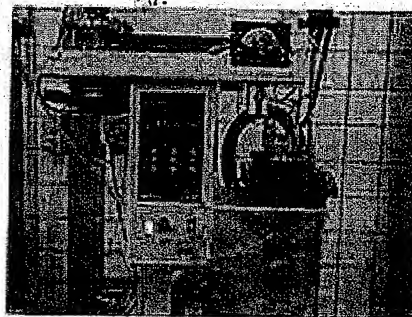
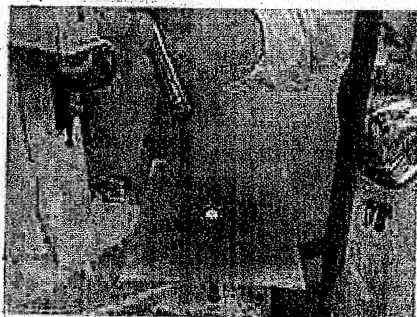


Fig. 3. The spray unit. The tablet is rotating with a speed of 40 rpm.

After spraying the tablets were placed in a temperature controlled cabinet for 3 minutes. 40°C for GRINDSTED® ACETEM 70-00 and 60°C for the bees' wax. This was done to secure a smooth and tight coating layer. Without this temperature treatment inspection in a stereo microscope showed scattered lumps of coating material on the surface of the tablets that has crystallised before reaching the tablet surface. After temperature treatment in the cabinets the tablet surface was smooth without any lumps. Finally the tablets were placed in 54% RH for minimum 2 days prior to measurement in the VTI.

The tablets were weighed just before the spraying and after reconditioning at 54% RH. The difference between the two weighings yields the amount of coating material applied on the tablets.

Adsorption isotherm for Ca-silicate:

Non-coated Ca-tablets were placed in 54% RH for 4 days prior to adsorption isotherm measurement. The measurement was done in VTI SGA-100 at 25°C with initial drying to constant weight with completely dry N₂. The equilibrium weight gain as a function of % RH was monitored. The slope of the linear part of the isotherm was calculated and used in equation 12 (b-value). This measurement needs only to be done once, but it must be redone if a new batch of Ca-silicate or a new batch of Ca-silicate tablets is used.

Sorption measurement:

Instrument: VTI SGA-100 symmetric sorption analyser.

The tablet was placed in the instrument using a small tablet holder that secured that moisture could be exchanged from all sides of the tablet.

The program sequence started with an initial drying with completely dry N₂ at 25°C. The dry weight was later used in eq. 12. After completely drying the next sequence was to equilibrate to 10% RH. 10% RH corresponds to the lowest water content in the tablet that is within the linear part of the adsorption isotherm for Ca-silicate and the water content at 10% RH yields m_i in equation 12.

After equilibration at 10% RH the humidity was increased to 80% in one step and the weight gain versus time was monitored.

Only one tablet can be measured per day due to the long equilibration time.

Calculation:

The calculation of the P/X-data from the raw data generated by the VTI-instrument was done by an Excel Macro: "Macro til normering af long data file 0-10-80". The macro recalculates the weight gain to be expressed on basis of the dry weight. The true dry weight of the coated tablets is the dry weight measured by the VTI instrument minus the weight of the coating on the tablets. The coating weight must be entered into the macro. Furthermore

the Macro plots the graph $LN \left[\frac{m_e + m_i}{m_e + m} \right]$ versus time in equation 12 using only data that is

within the linear part of the adsorption isotherm for Ca-silicate tablets. From the slope of the line the Macro calculates P/X using equation 12.

Self-supporting films (cup method):

Preparation of the film:

The coating material was made self-supporting by melting it on a piece of nylon filter, which can work as a stabilising network so the film formed could be handled in one piece without falling apart.

The film was prepared by placing the filter on a microscope object glass on which two bars equal in size was fixed as illustrated in fig. 4. The liquid coating material was applied on the filter and the second object glass was heated above the melting point of the coating material and gently placed on top of the first object glass with the coating in between. Special precaution was taken to avoid any inclusions of air bobbles. After crystallising the material both object glasses were gently slide of. The distance between the two object glasses could be varied in steps of 100, 200, 300, 400, 500 μ m.

This method was useful where the coating material did not adhered too strongly to the object glasses.



Fig. 4. Preparation of film between two object glasses.

If the coating material adhered strongly to the object glasses another method was used. The filter was then placed on the dull side of a piece of tinfoil situated on a heating plate with a temperature above the melting point of the coating. The fluid coating was applied on the filter and the tinfoil was moved to a flat and cold table for crystallisation after which the tinfoil was carefully removed.

The latter method did not secure as uniform film thickness as the first method.

Desorption measurement:

The filter with crystallised coating material was transferred to an alu-cup containing 25 droplets of de-ionised and de-aerated water as illustrated in fig.5. The cup had a small flange on which the filter could be placed and fixed with silicone grease:

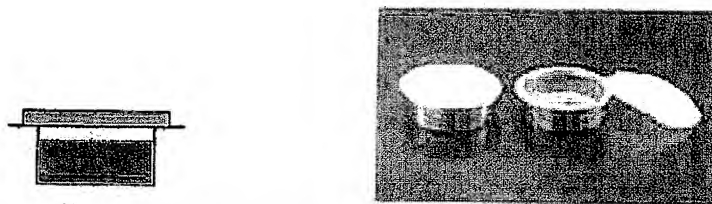


Fig. 5. Measuring cup with water and filter

Water evaporation secured a relative humidity of 100% in the head space.

The cup was placed in the VTI and the water loss was monitored in time at 25°C and 15% RH external relative humidity. The VTI set-up is stored in the VTI Template : **Plastic Polymer Film.**

The measuring time was 120 min, but elongated to 400 min for coatings with high resistance to water migration.

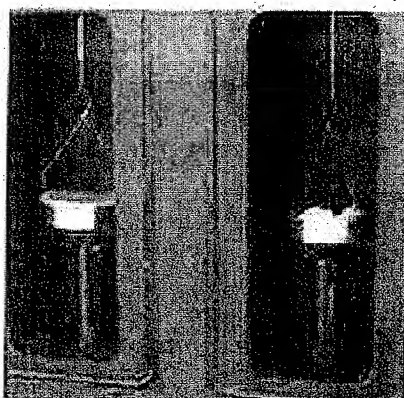


Fig. 6 Sample and contra weight hanging in the balance weight in the VTI.

Calculation:

The raw data file generated in the VTI was used to calculate P/X from equation 7 by use of an Excel Macro: **Macro till normering af VTI long data file for plastic film 15%.** The macro plots weight loss VS time and calculates the slope of the line, which was used to calculate P/X in equation 7. The Macro requires input about which cup has been used (cup 1 or 2), as the two cups differ in area. Cup 1 area = 4.08 cm² and cup 2 area = 4.31 cm².

Film thickness:

After measurement the filter was placed at 5°C for at least two hours after which the film thickness was measured with a micrometer 4 places on each filter. The values were averaged to provide the film thickness.

MATERIALS

GRINDSTED® ACETEM 70-00 lot 010364

Bees' wax:

Hvid bivoks blanding 150

vare nr. 1623150 batch.nr. 66438

Supplier: HCL NORDIC

Gl.Strandvej 16

DK-2990 Nivaa

Ca-silicate tablets. Prepared by Glostrup Apotek, Produktionsafdelingen. Phone: 43960010.
400 mg tablets produced without processing aid. Batch 0111061.

Ca-silicate powder: DC-product item no. 014520.

Filter: PW 1:1 0.055 mm SR 79T/200T Polyester

Bought through Udersens Grafisk Fagcenter A/S

RESULTS & DISCUSSION

The results of the VTI measurements are listed in appendix 2.

Spraying method:

Fig. 7 shows images from CLSM of GRINDSTED® ACETEM coated and non-coated Ca-silicate tablets. The focus layer is 20 µm below the surface of the tablets. The coated tablet contains 140 g/m² GRINDSTED® ACETEM 70-00.

The tablets were stained with Nile red dissolved in acetone by spreading a thin layer of the Nile red / acetone on a glass surface allowing the acetone to evaporate. The tablets were then placed on the glass surface for one day and evaluated.

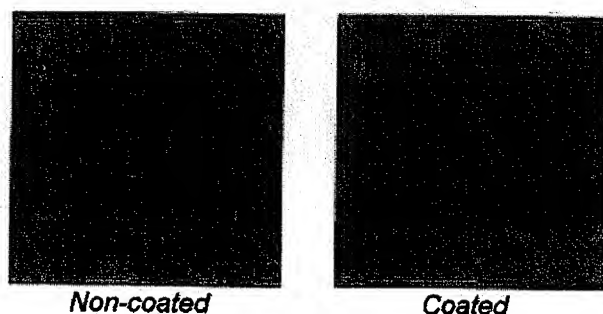


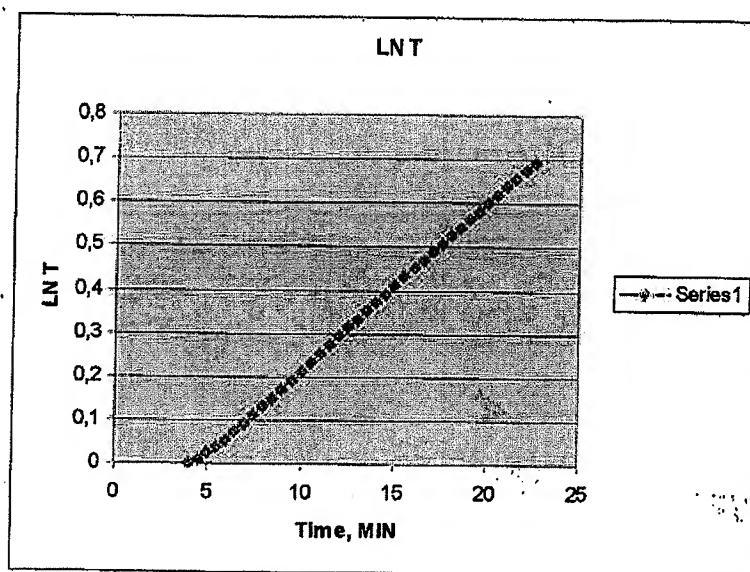
Fig. 7. Confocal microscopy of Ca-silicate tablets coated with 140 g/m² GRINDSTED® ACETEM 70-00 and stained with Nile red. Focus layer 20 µm.

The two images support the assumption that the coating layer penetrates the surface of the tablets in a more or less uniform way. The red dots on the non-coated tablet are Nile red crystals.

Appendix 3 provides the raw data generated by the VTI in graphic form. This raw data file is used to calculate the results shown in fig. 8, which is an example of calculating P/X using equation 12 for GRINDSTED® ACETEM 70-00 sprayed on Ca-silicate tablets.

$LN \left[\frac{m_e + m_t}{m_e + m} \right]$ in equation 12 is plotted against time. Time is set to zero when the relative

humidity changed from 10% RH to 80% RH. Note the very linear graph as predicted by eq. 12. The slope has positive sign as the tablets adsorb water during the measurement. The graph illustrates the difficulties in estimating the correct zero time (the graph is not passing through origin). This is however of no importance as only the slope of the line is used. Note that in this case the measurement only takes 20-25 min after equilibration at 10% RH. Adsorption continues after the 25 min, but at this stage the tablet has adsorbed so much water that the isotherm for Ca-silicate no longer follows the linear part as required in eq. 12. The Excel Macro discriminates the non-valid data.



Permeance \emptyset = 0,038629/MIN

Date 12-02-02

Kfilm = 293,8 mg H2O / m2 mmHg min

Film Weight = 6,4g/m2

Conditions: Tablet batch number = 0111061
 Temperature = 25°C
 Linear working isotherm, slope b = 0,030497 %W(DB)/ Aw
 Ktablet = 77,2mg H2O / m2 mmHg min

Fig 8. Calculation of P/X for GRINDSTED® ACETEM 70-00 sprayed on CA-silicate tablets

All P/X data are plotted against 1/X in fig. 9 for GRINDSTED® ACETEM 70-00 and fig. 10 for White Bee Wax. For GRINDSTED® ACETEM 70-00 the slope of the line equals P = 2520 mg g/m²/m² mmHg min. The correlation coefficient is fairly good.

**P/X VS 1/X for Acetem 70-00 at 25°C
Spray-method**

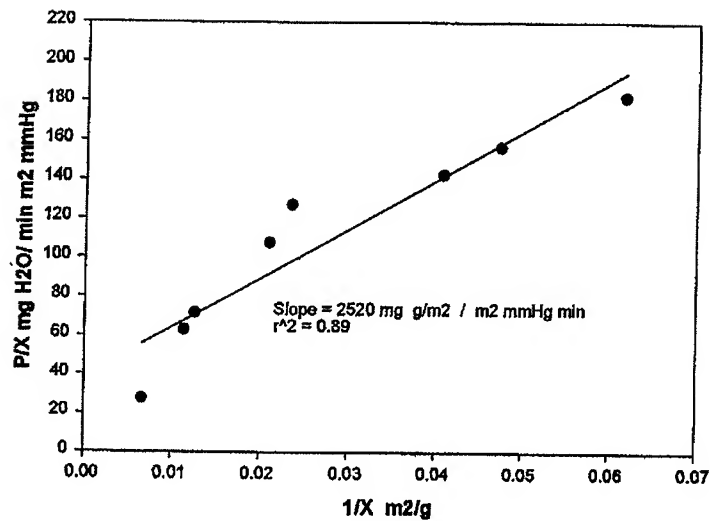


Fig. 9

**P/X VS 1/X for Bees Wax at 25°C
Spray method**

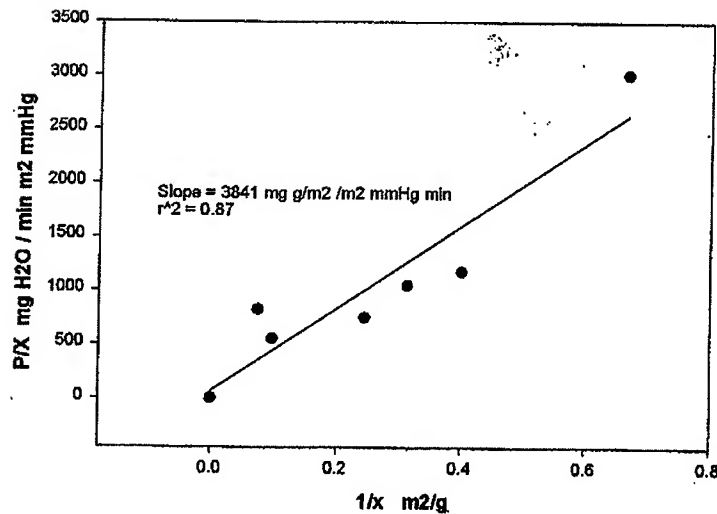


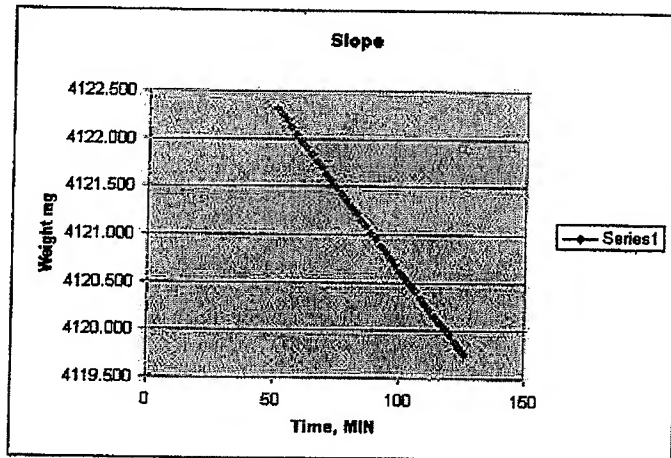
Fig. 10

The correlated P-value for White Bees' Wax is shown in fig. 10. With $P = 3841 \text{ mg g/m}^2 / \text{m}^2 \text{ mmHg min}$, the permeability is higher than that for GRINDSTED® ACETEM 70-00, a fact that might indicate the practical problems with using bees' wax that easily forms holes and cracks.

Cup method:

Fig. 11 illustrates an example of the measurement and calculation of the permeability of GRINDSTED® ACETEM 70-00 based on the cup method. With this method the sign of the

slope is negative as the samples evaporate water during measurement. The graph is very linear as expected from the theory.



Permeance -0.034mg H₂O / MIN

Date
13-02-02

Kfilm = 4.067 mg H₂O / m² mmHG min

Film Thickness = 311.00μm

Temperature = 25°C

Kw = 90.88mg H₂O / m² mmHg min

Fig. 11

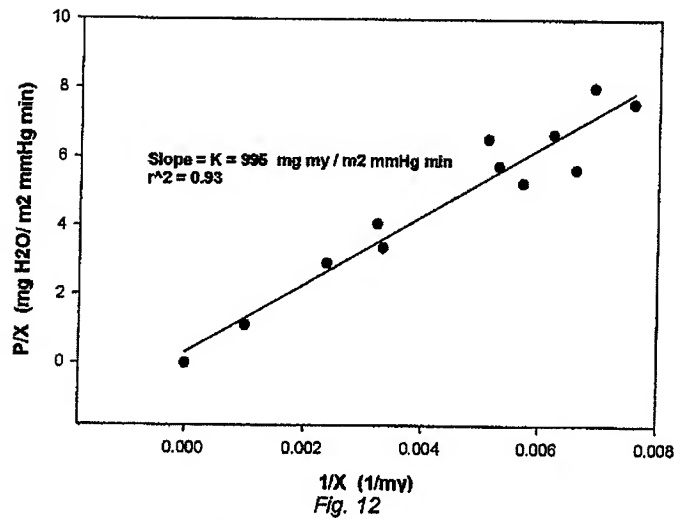
The calculated P/X for GRINDSTED® ACETEM 70-00 and White Bee Wax are plotted against the reciprocal film thickness and shown in figures 12 and 13.

White Bee Wax was prepared using both the object glass and the tinfoil methods. The wax adheres strongly to the glass and it tends to form lines or cracks when removed from the glass surface.

The P value of 186 mg μm / m² mmHg min is also significantly higher for this method compared with the tinfoil method, which was much easier to peel from the film. For this reason only 4 data points are included in the glass method for White Bee Wax.

The tinfoil method resulted in a much lower P value nicely located on a straight line. No cracks or lines were seen in the film with this method. The correlation coefficient is a disaster, which however is due to the very low value of P/X (0.007 – 0.133). The level is very close to the measuring limit of the method. The value of 0.007 corresponds to a weight loss of approx. 4 μg in one hour. The tinfoil method yields a P value of 14.1 mg μm / m² mmHg min.

**Permeability of Acetem 70-00 at 25°C
Glass method**



**Permeability of White Bee Wax at 25°C
Glass and Alu-foil Method**

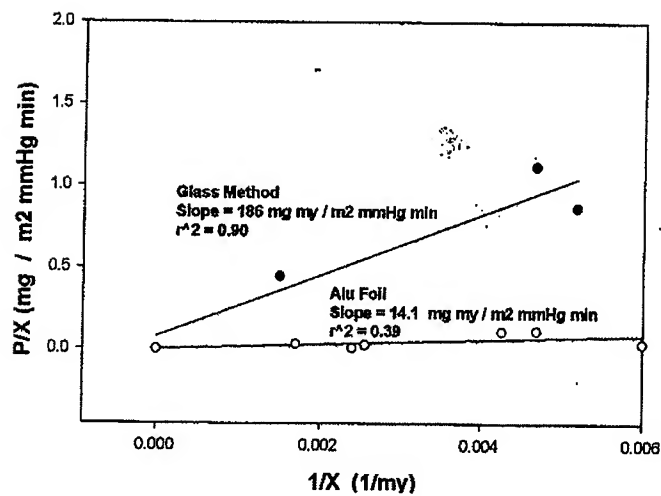


Fig. 13

Fig.12 shows the P/X plot for GRINDSTED® ACETEM 70-00 based on the glass method. GRINDSTED® ACETEM 70-00 did not adhere strongly to the glass surface and no cracks or lines were noticed in the films.

The correlation is good and the P value was calculated to $995 \text{ mg } \mu\text{m} / \text{m}^2 \text{ mmHg min}$.

Nanna Westergaard [9] reports the permeability for GRINDSTED® ACETEM 70-00 to $782 \text{ mg } \mu\text{m} / \text{m}^2 \text{ mmHg min}$ at 21.1°C (recalculated to same units). There is no information of the type of GRINDSTED® ACETEM, but as the permeability increases exponentially depending on the activation energy [4] with increasing temperature the data found for GRINDSTED® ACETEM 70-00 seems likely.

With $14.1 \text{ mg } \mu\text{m} / \text{m}^2 \text{ mmHg min}$ for white bee wax the permeability coefficient is approx. 70 times less than for GRINDSTED® ACETEM 70-00. J.J.Kester [5] reports 10 times less permeability through bee wax than through GRINDSTED® ACETEM, but Nanna Westergaard [9] lists a value of $1.74 \text{ mg } \mu\text{m} / \text{m}^2 \text{ mmHg min}$ at 25°C .

As expected the white bee wax resists water transportation much more effectively than GRINDSTED® ACETEM 70-00 when measured on a uniform and unbroken film.

The cup method provides a practically much easier approach compared with the tablet method and the results from the cup method can be considered as a material coefficient that depends only on the chemical and physical structure of the coating and not on how it is applied on the food. Furthermore it is much easier to avoid and control any holes or cracks in the coating layer.

The permeability data for the tablet method is significantly higher than for the cup method (note that the units differ for the two methods due to the different ways of measuring the film thickness. The conversion factor is however close to 1). One explanation is of course that the sprayed layer on the tablets is not uniform, but contains holes or cracks. Another possibility is that this reflects the practical permeability when the coating material is sprayed on a substance, where the coating is absorbed into the substance before crystallising.

Diffusion or film controlled

The above results have all been calculated on basis of equation 13, which compensates for the diffusion process.

The (P/X)t values for both the tablet and the cup are listed in appendix 2. They are measured on tablets without coating and the cup without filter.

(P/X)t for the tablet and the cup are 77.2 mg/m² mmHg min and 90.9 mg/m² mmHg min, respectively. Comparing the values with the (P/X)f values in appendix 2 shows that the water uptake in the tablets were both film and diffusion controlled during the measurement as the (P/X)t and (P/X)f values are at the same level. This is not the case for the cup method, where the (P/X)f values are much smaller indicating a true film controlled water evaporation.

The (P/X)t-value for the cup method is reliable as the cup area does not change when no filter is used.

This may not be the case for the tablet where the thru surface may differ from the surface area for a coated tablet. The coating may smoothen the surface. A non-coated tablet may therefore have a bigger surface area. The (P/X)t-value has therefore been calculated theoretically as shown below [3]:

$$\frac{B_D}{L_0} = \frac{\varnothing_{int} * 4 * L_0 * \rho * b}{\pi^2 * P_0}$$

B_D/L_0 : Permeability by diffusion (P/X)

\varnothing_{int} : Permeance (slope of the line according to eq. 12) = 0.0525/min

L_0 : half thickness of the tablets = 1.81 mm

ρ : density of the tablets = 1200 kg/m³

b : slope of the adsorption isotherm for the tablet = 0.03497.

P_0 : saturated water pressure at measuring temperature = 23.756 mmHg

Using above values yields a P/X-value for the diffusion of 59.4 mg/m² mmHg min. Although the calculated value is somewhat lower than the measured 77.2 mg/m² mmHg min, the difference is acceptable when comparing theoretical and measured data. The value of 77.2 mmHg/m² mmHg min has been used in the project.

PERSPECTIVE

The cup method is the most reliable method and will be used as the first choice. Up to 3 samples can be measured per day with the cup method and the method provides possibilities of testing a range of new coating developments relatively fast before going into application test. This means that a catalogue can be created with the permeability data listed as material coefficients as basic information for application trails, costumer contacts and coating development.

A:

For future tests the following combinations could be relevant:

1:

To improve the flexibility of bees' wax, GRINDSTED® ACETEM 70-00 could be mixed into the bees' wax in the right proportion, improving flexibility without losing the excellent water barrier properties.

2:

Dry ingredients could be mixed with GRINDSTED® ACETEM 70-00; e.g. hydrocolloids, finely ground sugar, salts, MCC, etc.

3:

Plastic non-polar fat such as Benefat could be tested alone or mixed with emulsifiers. Benefat is alpha stable, which should be the best crystal form to retard water migration.

4:

PLE could develop a non-polar, medium to high melting, alpha stable, physically elastic product, which from literature seems to provide the optimal barrier properties.

5:

Other plastizisers than GRINDSTED® ACETEM 70-00 could be added to bees' wax. Preferably non-polar components should be used.

B:

It is important to realise that when dealing with coating based on polar lipids the measured permeability coefficient is only valid for the specific relative humidity of the air on both sides of the film used during the measurement. Therefore it might be necessary to repeat the measurement for a particular coating material when it is applied at other humidity conditions than specified during the analysis. The relative humidity used in this project are 100% RH for the inner phase and 15% RH for the outer. The outer % RH can easily be adjusted by the VTI equipment. The inner % RH can be adjusted by using a salt solution instead of pure water.

C:

Furthermore, the test temperature also influences the permeability coefficient. 25°C was used in this project, but it might not be suitable for all food applications. The dependency of the permeability coefficients on the temperature follows the Arrhenius equation:

$$\frac{P}{X} = \left(\frac{P}{X}\right)_0 * e^{\frac{-E_a}{RT}}$$

Ea = activation energy, R = gas constant, T = Kelvin temperature, $\left(\frac{P}{X}\right)_0$ = permeability coefficient at 0K.

Above equation shows that measuring P/X at for instance four different temperatures and plotting $\ln(P/X)$ VS the reciprocal Kelvin temperature yields a straight line where the slope equals $\frac{-E_a}{RT}$ and the intercept $\ln\left(\frac{P}{X}\right)_0$. In principle, P/X for any relevant temperature can then be calculated without further measurements.

If the test temperature deviates from 25°C a new adsorption isotherm must be measured for Ca-silicate.

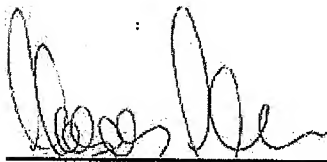
D:

Additional information regarding the flexibility and elasticity of the films should also be measured as this parameter has a big impact on the practical barrier property. This could perhaps be measured by Texture Analyser (fracture) or by rheology.

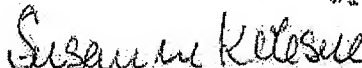
E:

Together with the measured permeability data, eq. 12 can also be used to predict the shelf life for a specific product or estimate the required performance of a coating layer. Knowledge about the food composition together with measurement of the adsorption or desorption isotherm for the food and specification of the storage condition, eq. 12 can inform about the required value of P/X . Knowing the permeability coefficient for a range of possible coating material the necessary thickness of the coating can be calculated. In that way both the applicative and the economical consequences can be estimated very early in a project.

$$\ln\left[\frac{m_a + m_t}{m_s + m}\right] = \frac{P}{x} * \frac{A}{Ws} * \frac{P_0}{b} * t \quad \text{eq. 12}$$



Mogens Nielsen
Senior Scientist



Susanne K. Olesen
Development Director

EXHIBIT D

REDACTED

Date

File No.

Metals given

Cup

Prove

Ref

02136	Plastic Polymer film, 5°C	2	Cellulose A	(1) 152529	Logans Creekman, 5°C	Yellow film	365
02137	"	"	"	"	"	"	372
02138	"	"	"	"	"	"	372
02139	"	"	"	"	"	"	372
02140	"	"	"	"	"	"	372
02141	"	"	"	"	"	"	372
02142	"	"	"	"	"	"	372
02143	"	"	"	"	"	"	372
02144	"	"	"	"	"	"	372
02145	"	"	"	"	"	"	372
02146	"	"	"	"	"	"	372
02147	"	"	"	"	"	"	372
02148	"	"	"	"	"	"	372
02149	"	"	"	"	"	"	372
02150	"	"	"	"	"	"	372
02151	Plastic Polymer film, 5°C	2	Cellulose A	(1) 152529	Logans Creekman, 5°C	Yellow film	365
02152	"	"	"	"	"	"	372
02153	"	"	"	"	"	"	372
02154	"	"	"	"	"	"	372
02155	"	"	"	"	"	"	372
02156	"	"	"	"	"	"	372
02157	"	"	"	"	"	"	372

stopped

Cellulose A 50% white film, 5°C, Yellow film

Date

REDACTED

File #	Metcalk name	Cup	Repts	Ref
02158	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4130
02159	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3826
02160	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	1030
02161	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3981
02162	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4090
02163	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4019
02164	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4197
02165	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3608
02166	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3320
02167	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4337
02168	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4300
02169	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4412
02170	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3831
02171	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3863
02172	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3890
02173	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3863
02174	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3738
02175	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	4081
02176	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3280
02177	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3415
02178	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3914
02179	Plastic polymer film, 5°C	2	white beeswax + 50% Acetone 70-00, 5°C, yellow film	3906

REDACTED

Date	File #	Metcell name	Cup	Notes	Rel
	02180	film, 5°C	2	White Resin + 80% Acetone 70°C, 5°C, yellow film	3415
	02181	-11-	2	-11-	2992
	02182	-11-	2	-11-	435
	02183	-11-	2	-11-	3815
	02184	-11-	2	-11-	3775
	02185	-11-	2	Acetone 70°C	383
	02186	-11-	2	-11-	361
	02187	-11-	2	-11-	1
	02188	-11-	2	-11-	3901
	02189	Des, 30°C/KH, 22°C	—	DAVE 22°C	—
	02190	-11-	—	2260/47	—
	02191	-11-	—	2260/45	—
	02192	fracture polymer film, 5°C	2	80% Acetone 70°C	3876
	02193	-11-	2	—	—
	02194	-11-	2	—	347
	02195	-11-	2	—	4264
	02196	-11-	2	—	3766
	02197	St. MNC	—	Control carbon	—
	02198	Plastic polymer film, 5°C	2	90% Acetone 70°C	3943
	02199	-11-	2	—	4002
	02200	-11-	2	—	3796
	02201	-11-	2	—	371

(Temp. 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, 100°C)

DATE	FIL NR	METHOD	CUP	PRGVE	REI
REDACTED					
	02203	phly, 5% -11-	2	90% Acetone -11-	354
	02204	-11-	2	-11-	3852
	02205	-11-	2	-11-	3891
	02206	-11-	2	95% - 5% -11-	367
	02207	-11-	2	-11-	3786
	02208	-11-	2	-11-	346
	02209	-11-	2	-11-	887
	02210	-11-	2	-11-	3845
	02211	-11-	2	-11-	4256
	02212	-11-	2	-11-	3712
	02213	-11-	2	-11-	3922
	02214	-11-	2	-11-	3577
	02215	-11-	2	-11-	424
	02216	-11-	2	-11-	4067
	02217	-11-	2	-11-	3892
	02218	-11-	2	-11-	4033
	02219	-11-	2	-11-	3798
	02220	-11-	2	100% Acetone -11-	419
	02221	-11-	2	-11-	3752
	02222	-11-	2	-11-	398
	02223	-11-	2	-11-	3954

Preparation of
sample filters

EXHIBIT E

From: "Mika Timmerbacka" <mika.timmerbacka@danisco.com>
To: <DAA@dvoung.co.uk>
Date: Redacted
Subject: new invention re: 0353 "Moisture barrier"

Hei David,

Enclosed is a short description of a new invention coming from Brabrand. We should file this soonest as customers are waiting for samples. What is your timetable? Maija-Liisa is trying to arrange meetings at Brabrand regarding mentioned new invention and another meeting regarding the plasticiser draft (our ref: 0259).

Parhai terveisin
Mika

(See attached file: Redacted)

This is an e-mail from Danisco and may contain confidential information. If you are not the intended recipient and you receive this e-mail by mistake, you are not allowed to use the information, to copy it or distribute it further. Please notify us and return it to Danisco by e-mail and delete all attachments. Thank you for your assistance.

CONFIDENTIAL

Our Ref: 0353

Enclosed is a short description of the invention

ACETEM (acetylated monoglycerides) has shown excellent properties as a plasticiser when mixed with beeswax. Beeswax is unique to prevent water migration if the beeswax film or coating is uniform and unbroken. However beeswax is very firm and brittle and it easily forms cracks and pinholes, which results in loss of barrier properties. Addition of up to 90% ACETEM improves the texture dramatically without alternating beeswax excellent resistance to water migration.

ACETEM itself has some resistance to water migration, but the key feature is the very plastic and elastic consistency.

Measurement of the permeability coefficient (P-value) shows that pure beeswax has a P-value less than 14 at 5°C and pure ACETEM approx. 750. A mixture of 80% ACETEM and 20% beeswax has a P-value of 27 (unit = mg μ m / m² mmHg min).

ACETEM plasticising properties is : Plastic/elastic
High solid content
Is compatibel with beeswax
Soften beeswax while keeping a high solid content in the mixture
Stable alfa-crystal form.

Redacted